REMARKS

The Office Action

Claims 1-38 are pending. Claims 1-6 and 25-38 stand rejected for lack of enablement. Claims 1-6, 25-32, and 35-38 stand further rejected for anticipation by Good et al. (U.S. Patent Publication No. 2002/0069423; hereafter "Good").

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-6 and 25-38 stand rejected for lack of enablement. As stated in M.P.E.P. § 2164, "[t]he purpose of the requirement that the specification describe how to make and use the claimed invention is to ensure that the invention is communicated to the interested public in a meaningful way." Applicants have met this standard.

In support of this rejection, the Office has asserted that producing transgenic mammals is generally unpredictable, relying on observations from sheep, goats, pigs, mice, and cows reported in Clark et al. (Transgenic Research 2000, 9:263), Denning et al. (Cloning and Stem Cells 2001, 3:221), and Dunne et al. (U.S. Patent Publication No. 2002/0194635). The Office also noted that only one transgenic bovine fetus was produced in the cited art and that the process was not reproducible, and further that work describe in the Denning et al. publication and a reference cited therein failed to produce a useful live-born calf. The Office further posits that prion protein knockouts in cows might produce adverse health effects based on mice studies. Finally, the Office states that

Applicants' methods are enabled for enucleated MII oocytes. Applicants address each of these concerns in turn below.

As stated in the enclosed Declaration of Dr. Yoshimi Kuroiwa, the specification provides ample guidance for one skilled in the art to produce hemizygous and homozygous prion protein (PrP) knockout cells and bovines reproducibly. The specification describes the sequence of the gene locus used to prepare a targeting vector, as well as the structure of a targeting vector including two homology regions, a transcriptional termination *(STOP)* sequence together with a drug-resistance gene flanked by two loxP sites, and a negative selection marker. The specification further describes general methods and provides specific conditions for transfection to produce first a hemizygous clone and then a homozygous clone. Finally, the specification provides methods for using the hemi- and homozygous clone cells to produce a bovine.

As further noted by Dr. Kuroiwa, the specification provides data showing that hemizygous $PrP^{+/-}$ cells were actually produced using the methods described in the specification at a frequency of approximately 50%, and homozygous $PrP^{-/-}$ cells were actually produced from hemizygous cells at a frequency of approximately 5%. These percentages are sufficient that one skilled in the art could replicate these results in 6 months using the disclosed methods.

In addition, the Declaration states that the methods described in the specification have been used to produce surviving hemizygous and homozygous PrP (also referred to as *PRNP*) knockout bovines. As stated by Dr. Kuroiwa, a male Holstein primary fetal

fibroblast line, 6594, was transfected with the first and second KO vectors (pBPrP(H)KOneo and pBPrP(H)KOpuro vectors) to sequentially disrupt the two alleles of the PrP gene. $PrP^{-/-}$ fetal cell lines were established at 40-60 days of gestation, and three of the $PrP^{-/-}$ fetal cell lines (5211, 5232 and 4296) were recloned to produce calves, yielding five (16 %), two (8.6 %), and five (26 %) calves from the cell lines 5211, 5232, and 4296, respectively.

In addition to the ability to produce living PrP knockout bovines, Dr. Kuroiwa further presents data on the health and reproductive capability of the PrP knockout bovines. As stated, $PrP^{-/-}$ cattle were monitored for growth and general health status from birth up to 18 months of age. Mean birth weight was 46 kg, and average daily gain was 0.91 kg/day to 10 months. Both values were in the normal range for Holstein bulls. Serum chemistry was evaluated at 6 months of age and compared with published reference ranges. All the values for $PrP^{-/-}$ calves (n = 12) were well within the reference range, and obvious abnormalities were not observed.

General physical examinations, done at monthly intervals by licensed veterinarians, included the following parameters: body temperature, heart rate, heart sound, jugular vein distension, respiratory rate, respiratory sound, presence of cough, nasal discharge, eye abnormalities, appetite, general behavior (alert and active, sluggish, hyperactive), gait, posture, joints, hooves, feces (diarrhea, constipation), genitalia, and umbilical cord (dry, enlarged, inflamed, infected). All parameters were normal for all

$PrP^{-/-}$ cattle (n = 12), and all $PrP^{-/-}$ cattle survived this observation period without any unusual health problems.

At 10 months of age, eight pairs of $PrP^{-/-}$ and age, sex, and breed-matched wild type control cattle were further given an extensive clinical examination (consisting of 122 parameters). These examinations were done according to the diagnostic evaluation of ruminants suspected of TSE (transmissible spongiform encephalopathy) as described in the European TSE guideline "Surveillance and diagnostic of TSEs in ruminants". The clinical evaluation included a general examination of all organic systems and a detailed examination of the nervous system. Examination of the nervous system focused on the following aspects: (i) evaluation of mental status, studied by observation of animal behaviour and reactions to stimulations (approaching, menace, sounds, and light); (ii) evaluation of sensory function in limbs and trunk that included the study of superficial sensitivity, medular reflexes, and conscious proprioception; (iii) evaluation of motor function in limbs and trunk by studying the muscular tone, motor irritability (presence of muscles fasciculation and tremor), and gait abnormalities; and (iv) evaluation of cranial nerves that was done by the observation of disorders in the corresponding innervated regions. All animals ($PrP^{-/-}$ and controls cattle) showed a good healthy status in the general clinical examination, and none showed significant alterations in the examination of the nervous system, except the following observation; a mild increased reaction to external stimulation (menace and sounds) was observed in 3/8 $PrP^{-/-}$ cattle compared to 1/8 control cattle. The response to external stimulation was recorded as positive when it

was observed in three consecutive stimulations. This observation does not indicate an alteration of nervous system but only a mild nervous temperament, which was mainly observed in the knockout cattle.

Blood samples were taken for hematology from five pairs of $PrP^{-/-}$ and control cattle at 10 and 12 months of age. The means for various hematological parameters from the two samples were compared between $PrP^{-/-}$ and control cattle and with published reference ranges. Overall, hematology analysis did not reveal obvious unusual characteristics in $PrP^{-/-}$ cattle at 10 or 12 months of age.

To evaluate the impact of PrP^{C} deletion on calf development, extensive histopathological analyses was performed on two $PrP^{-/-}$ and two wild type cattle at 14 months of age. Representative samples of skin, nasal turbinate, lung, liver, kidney, spleen, salivary gland, thyroid gland, tonsils (pharyngeal, palatine), thymus, reticulum, rumen, omasum, abomasum, intestines (ileum, colon), adrenal gland, pancreas, urinary bladder, lymph nodes (retropharyngeal, prescapular, mesenteric, popliteal), aorta, striated muscles (heart, tongue, masseter, diaphragm, triceps, psoas major, biceps femoris), testicle (from 2 animals), nictitating membrane, sciatic nerve, both trigeminal nerves and ganglia, pituitary gland, spinal cord (cervical, thoracic, lumbar), one eye with its optic nerve, and the whole brain were evaluated. At least 14 sections of various areas of the brain (including obex, pons, colliculi, cerebellum, hippocampus, thalamus, and cerebral cortex) of each animal were examined by light microscopy. Two sections of spinal cord at cervical, thoracic, and lumbar regions were also evaluated by light microscopy.

Neither obvious abnormalities nor significant lesions were observed for any tissue in either of the two groups.

Cells of the immune system play an important role in the pathogenesis of prion diseases, and PrP^C expression is readily detected in immune cells. No differences were observed in any of the cell subsets tested between $PrP^{-/-}$ and wild type cattle. To address immune-competence, $PrP^{-/-}$ cattle were immunized with ovalbumin (OVA), a T cell-dependent antigen. Statistical analysis using Student's t test showed no significant difference between $PrP^{-/-}$ and WT cattle ($p = 0.9$)

As stated by Dr. Kuroiwa, it has been shown in *Prnp*$^{-/-}$ mice that T cell proliferation and cytokine production induced by T cell mitogens is significantly affected, suggesting a role of PrP^C in T cell function. Therefore, PBLs were isolated from $PrP^{-/-}$ cattle and stimulated with anti-CD3 antibody, concanavalin A (ConA), and phytohemagglutinin (PHA). Statistical analysis using Student's t test showed no significant difference between $PrP^{-/-}$ and WT cattle ($p = 0.9$ for anti-CD3; $p = 0.4$ for Con A and $p = 0.7$ for PHA). In contrast to *Prnp*$^{-/-}$ mice, no difference in T cell proliferation after T cell mitogen stimulation was observed for $PrP^{-/-}$ cattle when compared to similarly treated cells from wild type cattle. PBLs were also stimulated by immobilized anti-CD3 monoclonal antibody for 72 hours, and intracellular IFNγ production was analyzed by surface CD3 and intracellular IFNγ (green) dual color immunofluorescent staining. PBLs isolated from four $PrP^{-/-}$ and four WT cattle were stimulated by (i) immobilized anti-CD3 monoclonal antibody or (ii) Con A for 72 hours

and secreted IFNγ in the culture supernatant was analyzed by calibrated bovine IFNγ ELISA. Statistical analysis using Student's t test showed no significant difference between $PrP^{-/-}$ and WT cattle ($p = 0.5$). These data indicate that the ablation of PrP^{C} expression does not appear to have deleterious effects on the immune systems in cattle.

With respect to the reproductive capability of the bovines, the $PrP^{-/-}$ bulls reached sexual maturity at a normal age, and semen was collected from two KO animals at 16 months of age to address fertility of $PrP^{-/-}$ cattle. Sperm appeared morphologically normal and were capable of generating normal-appearing blastocysts by *in vitro* fertilization with oocytes derived from wild type cows. This result indicates that $PrP^{-/-}$ bulls are reproductively normal and could be used for production of a population of $PrP^{-/-}$ cattle for commercial application.

Collectively, these data indicate that disruption of the normal prion protein PrP^{C} expression in *Bos taurus* does not adversely affect in any significant way normal bovine development. $PrP^{-/-}$ cattle remained healthy for at least 18 months after birth.

In sum, using the methods described in the present specification, it is possible to reproducibly produce PrP knockout cells and bovines, and further these bovines are healthy and survive to a useful age. Thus, the claimed bovines, cells, and methods of producing cells and bovines are predictable and enabled.

As the Office has raised a specific concern over the type of oocyte recited in claim 37, this claim has been amended to recite that the oocyte is an enucleated metaphase II oocyte. The rejection for lack of enablement should be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 1-6, 25-32, and 35-38 stand further rejected for anticipation by Good. "The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation" (M.P.E.P. § 2121.01; citations omitted). Moreover, "[w]hen a prior art reference merely discloses the structure of the claimed compound, evidence showing that attempts to prepare that compound were unsuccessful before the date of invention will be adequate to show inoperability" (M.P.E.P. § 2121.02; citations omitted). The anticipation rejection in this case should be withdrawn because Good is not an enabling disclosure.

Good tried and failed to produce even a hemizygous prion protein knockout cell. For example, as indicated in Good, the intent was to isolate the bovine PrP genomic fragment by screening a bovine genomic library using a DNA probe prepared from a mouse PrP gene. As stated by Good, "[n]one [of the genomic fragments identified] contained sequences of PrP which could be used to construct a targeting vector." (page 12, paragraph 0122). Thus, Good failed in a first attempt even to obtain genomic fragments to begin construction of a targeting vector. In a second attempt, Good decided to amplify the bovine PrP genomic fragment by PCR and constructed a single KO vector. That vector is shown in Fig. 5 of Good. Good attempted to combine this vector with genomic DNA. As stated by Good, "it failed." (page 12, paragraph 0126). This vector is the only example that was actually used in an attempt to produce PrP knockout cells.

Good failed to produce any experimental evidence that the methods described were operative. Indeed, both actual experiments described in the application were failures. In addition to these failed methods, Good provides a prophetic general plan for producing a prion protein knockout in Example 2; however, there are no data to suggest that this proposed plan is operable, which would be especially important given the previous failures. The case law is clear that failure to produce a product is adequate to show inoperability. In this case, Good tried and failed twice to produce even a knockout cell, much less a surviving bovine. Thus, the methods disclosed in Good for producing a prion knockout bovine do not anticipate the instant claims.

In addition to these legal arguments, Applicants submit a declaration by Dr. Kuroiwa with respect to the inoperability of Good's prophetic method for producing a prion knockout bovine. As is stated by Dr. Kuroiwa, the prophetic examples provided by Good are insufficient for one skilled in the art to produce a prion protein knockout. Furthermore, Dr. Kuroiwa's opinion is that the Good disclosure, when considered alone, indicates that prion knockout cattle could not be generated.

As stated by Dr. Kuroiwa, Good failed to provide any information in paragraph 0156 on how to determine whether the bovine PrP genomic DNA fragment shown in Fig. 12 would be isolated from the genomic library in a form capable of being successfully employed in a targeting vector. In the art, restriction map information and partial sequence information would be important to exploit an isolated genomic DNA fragment.

Furthermore, the complexity of the genomic library and the number of individual phage to be screened will determine whether the genomic DNA fragment can be isolated. As the genomic DNA fragment necessary for vector construction was not isolated from the genomic library using a ^{32}P-labeled mouse PrP probe in paragraph 0122, it is uncertain whether the genomic DNA fragment could be successfully isolated using the same genomic library and a non-isotopic probe, as proposed in EXAMPLE 2. Generally, a ^{32}P-labeled probe is more sensitive than a non-isotopic probe. Thus, one skilled in the art would be unsure whether the method described by Good would succeed.

Good further fails to provide any specific guidance in paragraph 0158 on how to construct the targeting vector shown in Fig. 12. The feasibility of constructing the vector as envisioned will depend on the genomic DNA fragment that might be isolated and the kinds of restriction enzymes that will be available for modification of the genomic DNA fragment. Since successful construction of a targeting vector is critical to the success of Good's methods, specific information of how to construct the targeting vector should be provided. A third party could not readily build the vector proposed by Good because of this lack of information on how pieces of the vector would be joined.

Finally, Good fails to provide information on the structure of the targeting vector just before the electroporation in paragraph 0165. For example, no guidance on whether the vector is in circular or linear form is provided, and, if the form is linear, no information is provided on how to linearize the vector. Because circular vectors are not integrated as efficiently as linear vectors, one skilled in the art should be provided with

information on both the form of the vector and the conditions under which it is to be transfected into a cell. Consistent with Dr. Kuroiwa's view, the Office rejected the claims in the Good application for lack of enablement (U.S. Application No. 09/816,546 and its continuation 10/971,541).

The processes described in the present application are distinct from those of Good because the present application allows one skilled in the art to produce the inventions instantly claimed. One key distinction over Good is that, as discussed above, the methods described in the application have been successfully used to produce PrP knockout cells and living bovines. In addition, the specification describes the identification of a genomic PrP DNA fragment (page 46, lines11-17 and page 55, lines 13-20) and provides exemplary targeting vectors and methods for their construction (page 46, lines 17-30, page 47, lines 12-23, and page 55, lines 20-30), structural information on the vector and methods of bovine cloning (page 55, line 30 – page 56, line 26 and page 58, line 28 – page 59, line 19), and a diagnostic PCR assay to genotype the cells after drug selection (page 56, line 28 – page 57, line 25 and page 59,line 21 – page 61, line 17). Finally, as stated by Dr. Kuroiwa, one skilled in the art could reproducibly produce PrP knockout cells and bovines using the methods of the present application.

In sum, Good is not an enabling disclosure, and the anticipation rejection should be withdrawn.

CONCLUSION

Applicants enclose a check for $50.00 in payment of the excess claims fee.

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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